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PENSOFT

Discovery of *Aedes* (*Ochlerotatus*) *pionips* Dyar, 1919 (Diptera, Culicidae) in Germany

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Abstract

A group of black-legged mosquito species within the subgenus *Ochlerotatus* Lynch Arribalzaga, 1891 of the genus *Aedes* Meigen, 1818 is difficult to identify based on their morphology. This group includes *Aedes pionips* Dyar, 1919, whose first record in the Erzgebirge (Germany) is reported in this study. The objective was to compile and add species specific characters for the morphological discrimination of *Ae. pionips* from similar mosquitoes in order to facilitate species identification. Generated cytochrome c oxidase subunit I gene DNA sequences of *Ae. pionips* from Germany were compared to sequences from related species, using a phylogenetic tree.

Keywords

Distribution, first record, morphology, mosquito, Ore Mountains, peatland, rare

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Introduction

Despite ongoing efforts to monitor the occurrence of mosquitoes in Germany, habitats such as peatlands, which can be sanctuaries for rare mosquito species, are poorly surveyed. Owing to the importance of such habitats for ecosystem functioning, the motivation of this study was to search for rare mosquito species in peatlands of the Erzgebirge (Ore Mountains, English; Krušné hory, Czech) in Germany. This search led to the first discovery of the mosquito species *Aedes* (*Ochlerotatus*) *pionips* Dyar, 1919 in Germany, and its taxonomic characteristics are described here.

The mosquitoes which are usually reported in Holarctic peatlands belong to a group of black-legged species of the subgenus *Ochlerotatus* Lynch Arribalzaga, 1891 within the genus *Aedes* Meigen, 1818 (Mohrig 1969). This group includes *Ae. pionips*, which is a Holarctic species that occurs in northern coniferous and boreal forests (Rempel 1953; Wood et al. 1979). *Aedes pionips* is distributed in North America (Alaska, Canada, northwestern United States), as well as in the region from northern Europe to the Kamchatka Peninsula and south to Kazakhstan and the Inner Mongolia Autonomous Region (Dubitsky 1970; Gutsevich et al. 1974; Tian 2009; Wood et al. 1979). In Europe, *Ae. pionips* has been recorded in Belarus, European Russia, Finland, Montenegro, Norway, Poland, and Sweden (Dahl 1974; Gutsevich et al. 1974; Mehl et al. 1983; Robert et al. 2019; Suslo 2020; Utrio 1975; Wegner 1991).

Larvae of *Ae. pionips* have been reported from a variety of aquatic sites, ranging from snowmelt pools to flooded grasslands (Culverwell et al. 2021; Wood et al. 1979). These larval habitats comprise *Sphagnum*-lined

pools, ditches, tracks, bog depressions, oxbow lakes, extensive deep ponds, and borrow pits (Carpenter and LaCasse 1955; Gjullin et al. 1961; Jenkins and Knight 1952; Wood et al. 1979). Larvae were found in open areas (e.g. *Sphagnum*-heath bogs: Jenkins and Knight 1952) and boggy forests (spruce, small leaved or pine forests: Khalin and Aibulatov 2019) from plains to mountain landscapes that rise to high altitudes (up to 915 m: Carpenter and LaCasse 1955; between 2400–3200 m: Denke et al. 1996).

Larvae of this monocyclic species hatch from overwintering eggs in spring (Carpenter and LaCasse 1955; Gutsevich et al. 1974) and have been observed between 1 May and 15 June (Jenkins and Knight 1952; Khalin and Aibulatov 2019). In general, *Ae. pionips* develops slowly, and adults appear between mid and late June, which is later than described for associated mosquito species (Dyar 1920; Gjullin et al. 1961; Belova et al. 2008). In the northwestern region of Russia, adults of *Ae. pionips* have been collected between 25 May and 3 August (Khalin and Aibulatov 2019).

The females of *Ae. pionips* are morphologically similar to *Ae. communis* (De Geer, 1776) and *Ae. punctor* (Kirby, 1837) but are larger and more intensely coloured (Dyar 1919; Gutsevich et al. 1974). However, these features alone are not sufficient for reliable identification (Vockeroth 1952). Characteristic features of *Ae. pionips* females include the colour of the scales on the scutum, the postpronotum, the terga and the wings, as well as the presence of scales on the postprocoxal membrane (Carpenter and LaCasse 1955; Dyar 1919; Gjullin et al. 1961; Gjullin and Eddy 1972; Gutsevich et al. 1974; Vockeroth 1952, 1954; Wood et al. 1979).

The morphological differences described for male hypopygia of *Ae. communis* and *Ae. pionips* are regarded as very minor, apparently within the range of intraspecific variation, so that males of both species are considered to be nearly indistinguishable by these characters (Gjullin et al. 1961; Gutsevich et al. 1974; Wood et al. 1979). The few discriminating features described in males include the shape of the gonocoxite, the shape and number of setae on the basal dorsomesal lobe, the shape of the apicodorsal lobe and its setae, and the length and shape of the palps (Dyar 1919; Vockeroth 1952; Carpenter and LaCasse 1955; Gjullin et al. 1961; Danilov 1984; Becker et al. 2020).

The fourth-instar larva of *Ae. pionips* is large and dark and can be clearly identified by the characteristics of the antennae, the cranial setae (5-C, 6-C, and 7-C), the thoracic setae (1-P, 1-M, and 3-M), and the saddle spines, as well as the number and shape of the comb scales (Frohne 1955; Gutsevich et al. 1974; Jenkins and Knight 1952; Rempel 1950; Vockeroth 1952; Wood et al. 1979). These features are helpful for distinguishing *Ae. pionips* from the morphologically similar larva of *Ae. pullatus* (Coquillett, 1904).

Mammals are the preferred source of blood for *Ae. pionips* (Dubitsky 1970; Schäfer and Lundström 2001). It is not known if *Ae. pionips* is a vector for pathogens (Kampen and Walther 2018), but Bassett (2014) did not exclude its involvement in the transmission of Snowshoe Hare virus.

Taken together, the morphological identification of *Ae. pionips* is currently hampered by incomplete identification keys, since information on morphological characteristics are scattered throughout the literature. The objective was to compile and add morphological characters of *Ae. pionips* in order to improve species identification. The collection sites as well as the morphological characteristics of *Ae. pionips* from Germany are discussed and compared to the morphologically similar *Ae. communis*, *Ae. pullatus,* and *Ae. punctor*, which were found in this study at the same collection sites. In addition, generated cytochrome c oxidase subunit I (COI) gene DNA sequences of *Ae. pionips* from Germany were compared to sequences from related species and discussed, using a phylogenetic tree.

Methods

Various pools were surveyed for mosquito larvae in the vicinity of the Georgenfelder Hochmoor, which is located near Zinnwald-Georgenfeld in the eastern Erzgebirge (federal state of Saxony, Germany), on the border with the Czech Republic. Mosquitoes of different developmental stages were collected on 19 June and 18 July 2021 in two neighbouring pools, as well as in a clearing in a spruce forest (potential natural vegetation: *Vaccinio uliginosi-Piceetum*; habitat type 91D4 according to Natura 2000: mire spruce woods), west to the Georgenfelder Hochmoor (European Commission, DG Environment 2007; Freistaat Sachsen et al. 2006; Fig. 1). The first pool, sampled in June, was a mossy bog pond with a saturated *Sphagnum* mat and Blue Spruce branches (*Picea pungens* Engelm.), located at the eastern forest edge near the ditch Neugraben (50.730°N, 013.739°E; Fig. 2). The second pool, sampled in July, was a vegetation-free pond with Norway Spruce branches (*Picea abies* (L.) H. Karst.) at a forest clearing (50.729°N, 013.738°E; Fig. 3). Moreover, the water temperatures of the aquatic habitats were measured with a precision laboratory thermometer.

Larvae were collected using a strainer at various sites of the pools, not following a specific sampling regime. They were reared at room temperature of 19–21 °C in jars containing water and soil substrate from the larval habitats. Samples from June were placed at room temperature and not cooled, whereas samples from July were placed in a water bath and cooled down once a day to 14 °C with an ice pack. As soon as the temperature of the water bath reached 14 °C, the ice pack was removed, and the temperature was allowed to rise to room temperature.

Adults were caught with an insect net in the vicinity of the first pool in June as well as of a forest clearing in July (50.729°N, 013.736°E). The mosquitoes were caught when they approached or were startled out of the vegetation. Caught adults were killed immediately with

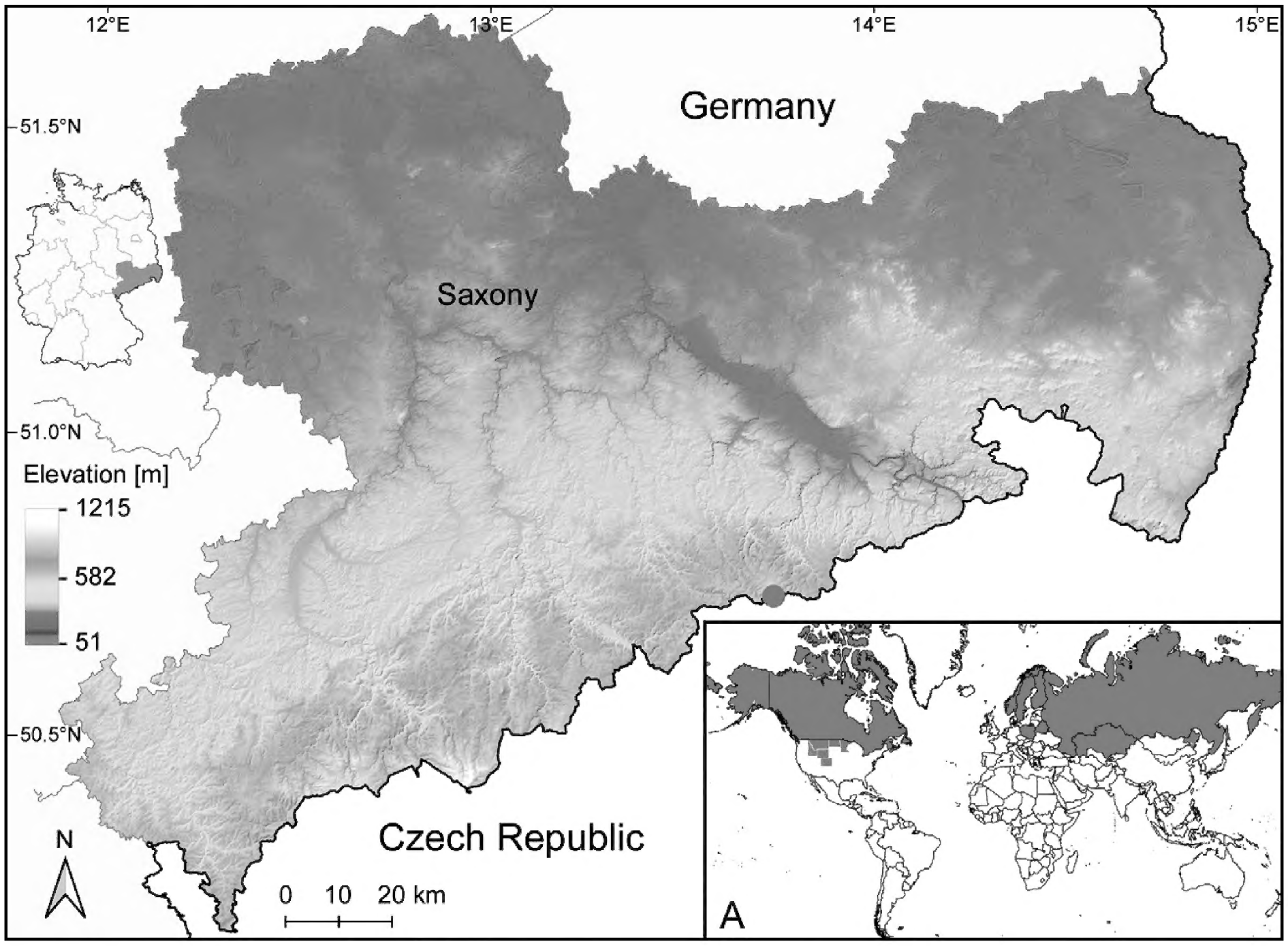


Figure 1. Map of the federal state of Saxony, Germany, with the sampling locations of *Aedes pionips* (red dot) (Map source: NASA SRTM 2013). **A.** Previously known range of *Aedes pionips* (red areas).

Figures 2, 3. The two larval habitats of *Aedes pionips* in a mire spruce wood. **2.** A bog hollow sampled in June 2021. **3.** A dystrophic pond sampled in July 2021.

ethyl acetate. Adults emerging from larvae were killed by overnight freezing (–20 °C). The killed adults were dry-pinned on minuten. Larvae that did not complete their development were stored in pure ethanol for further identification.

Mosquito specimens were morphologically identified using the keys of Becker et al. (2020), Gunay et al. (2020), and Wood et al. (1979), as well as remarks by Vockeroth (1952) and Danilov (1984). The identification was performed under a Leica M125 C stereomicroscope (100× maximum magnification) and images were taken with a Leica MC170 HD microscope camera. For long-term conservation, the specimens were stored in the author's private mosquito reference collection. For comparisons of morphological characteristics, additional mosquito specimens, which were collected at other sites in Saxony in 2019, were used from the author's private mosquito reference collection. The abbreviation "cf." (conferre), as a sign for open naming, was used when the determination remained uncertain because of diagnostic features

that were atypically developed or missing in damaged specimens.

One collected female and male, unambiguously identified morphologically as *Aedes pionips*, were selected for molecular characterisation of the COI barcoding region. A fragment of the right hindleg of each mosquito was cut-off using a sterile blade and placed in an Eppendorf tube for DNA extraction. Genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's instructions. The Folmer fragment, or "barcode fragment", which is the 5′ region of the COI gene, was amplified using a standard polymerase chain reaction (PCR) protocol with the forward primer LCO (5′-GCTCAACAAATCATAAAGATATTGG-3′) and the reverse primer HCO (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al. 1994). PCR amplification was conducted in a thermocycler with an initial denaturation step of 95 °C for 5 min, followed by 40 cycles of 94 °C for 45 s, 48 °C for 45 s, 72 °C for 1 min, and one cycle at 72 °C for 10 min. The PCR amplicons were sequenced using the same primers and the Big Dye Terminator version 1.1 cycle sequencing kit (Applied Biosystems). Sequences were edited using the BioEdit version 7.2.6.1 and compared with sequences deposited in the GenBank DNA sequence database (National Center for Biotechnology Information) and the Barcode of Life Data Systems (BOLD).

To construct a phylogenetic tree, publicly available COI sequences of *Ae. pionips* and related species were downloaded from the GenBank DNA sequence database and the Barcode of Life Data Systems. Sequences were aligned with ClustalO and trimmed to the same length of 618 base pairs using the program MEGA X (Kumar et al. 2018). The neighbour-joining method was used to infer the evolutionary relationship of the species. The validity of the clusters was tested with the bootstrap method supported by 1,000 replicates.

Results

Larvae of *Aedes pionips* were found in two water-filled depressions that resulted from peat sagging (Freistaat Sachsen et al. 2006). In the first pool, which was covered by moss, seven out of 39 larvae were morphologically identified as *Ae. pionips* (Table 1). Associated species were *Ae. communis*, *Ae. pullatus,* and *Ae. punctor*, which were also collected as adults next to the pool. In the second pool, which was free of vegetation, 20 out of 54 larvae were morphologically identified as *Ae. pionips*. In this larval habitat, larvae of *Ae. pionips* were associated with those of *Ae.* cf. *communis*, *Ae. pullatus*, and *Ae. punctor*. The four emerged males of *Ae.* cf. *communis* had similar genital characteristics as the males of *Ae. pionips* but lacked the postprocoxal scales. As the scales could have been lost, it is possible that these specimens belonged to *Ae. pionips*, meaning that no *Ae. communis* larvae were found in this larval habitat. Several *Ae. pionips* females were observed in the afternoon when they flew into a sunlit forest clearing. The females were hesitant when approaching or probing the skin, which was similarly observed by Rempel (1953). Before sunset, the approach rate increased, but the observed females always occurred as single individuals. Females of *Ae. pionips* were quickly recognisable by their body size, which was large when compared to other mosquito species. Co-occurring species that approached or rested in the vegetation included *Ae. communis*, *Ae. pullatus*, *Ae. punctor*, *Ae. sticticus* (Meigen, 1838), and *Ae. vexans* (Meigen, 1830) (Table 1).

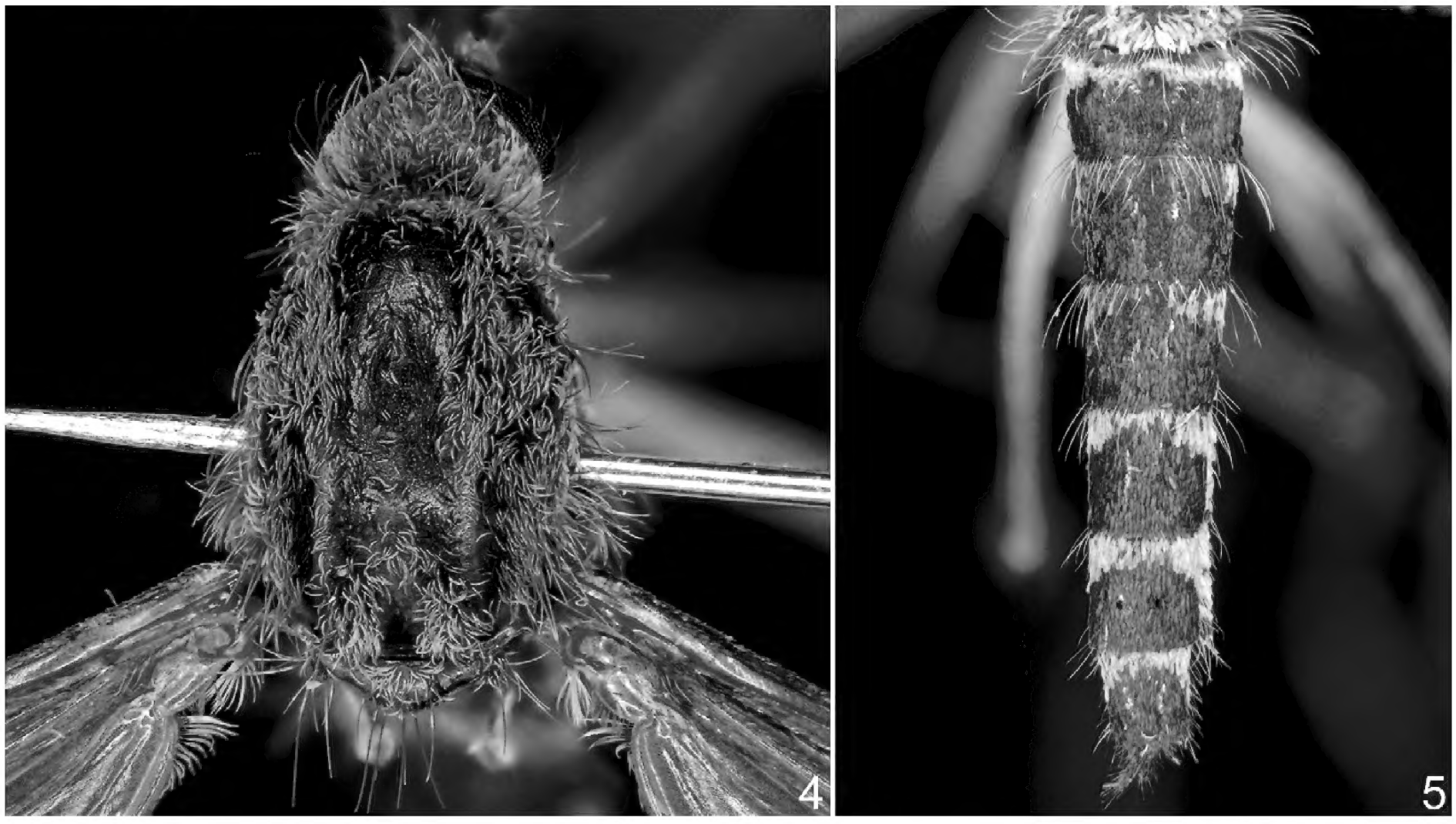
First records. GERMANY – **Saxony** • Erzgebirge, Zinnwald-Georgenfeld, west to Georgenfelder Hochmoor; 50.730°N, 013.739°E; 839 m alt.; 19.VI.2021; Cornelius Kuhlisch leg.; bog pond, strainer; 7 larvae, sex indet., 96% pure ethanol • same locality; 50.729°N, 013.738°E; 837 m alt.; 18.VII.2021; Cornelius Kuhlisch leg.; bog pond, strainer; 1 ♂, PCKU-DE-96, GenBank: OM349597; 8 ♂, 10 ♀, 1 larva, sex indet., dry and 96% pure ethanol • same locality; 50.729°N, 013.736°E; 837 m alt.; 18.VII.2021; Cornelius Kuhlisch leg.; forest clearing, insect net; 1 ♀, PCKU-DE-94, GenBank: OM349596; 2 ♂, 3 ♀, dry and 96% pure ethanol.

Identification. *Aedes pionips* specimens were distinguishable from co-occurring specimens of *Ae. communis* and *Ae. punctor* by the postprocoxal scale patch and the scale pattern on the scutum, postpronotum and tergum in females (Figs. 4, 5); and by the setae on the basal dorsomesal lobe of the gonocoxite, the claspette filament, and the postprocoxal scales in males.

To differentiate *Ae. pionips* from *Ae. communis* in the adult stage, it was helpful to compare the colour of the

Table 1. Numbers of larvae and adults of mosquito species collected at and next to the two pools and at the forest clearing near the Georgenfelder Hochmoor. In brackets: numbers of the collected or emerged males (m) and females (f); * hypopygia were similar to *Aedes pionips* males but postprocoxal scales were not present.

Sampled species	Pool 1 (19-06-2021)		Pool 2 (18-07-2021)	Forest clearing (18-07-2021)
	Larvae total (m, f)	Adults total (m, f)	Larvae total (m, f)	Adults total (m, f)
Aedes communis	2 (2, 0)	3 (0, 3)	4* (4*, 0)	3 (1, 2)
Aedes pionips	7 (0, 0)	—	20 (9, 10)	6 (2, 4)
Aedes pullatus	9 (1, 2)	4 (0, 4)	29 (19, 10)	3 (0, 3)
Aedes punctor	21 (1, 17)	27 (0, 27)	1 (0, 1)	7 (2, 5)
Aedes sticticus	—	—	—	1 (0, 1)
Aedes vexans	—	—	—	9 (3, 6)
Total	39	34	54	29



Figures 4, 5. *Aedes pionips* female. **4.** Dorsal view of the thorax. **5.** Dorsal view of the abdomen.

scutal scales, which was distinct in the collected specimens in good condition (Table 2). There were a few difficulties in the differentiation of certain *Ae. communis* specimens, which resembled *Ae. pionips*. In a dark colour variant of *Ae. communis* from the Erzgebirge (30 km northwest of the collection site, 450 m alt.), the usually pale acrostichal stripe was very dark with few pale scales, so that it appeared narrow and very indistinct. Furthermore, *Ae. communis* females from lower landscapes of Saxony (Moritzburg lake area, 50 km north of the collection site, 180 m alt.) had a lighter body scale colouration than specimens from collection sites at the Erzgebirge. Thus, the dark scutal stripes of the specimens from the Erzgebirge appeared distinct and contrasted with the pale scales on the scutum. Other characters of *Ae. communis* were as typically described.

The scales on the postpronotum of *Ae. pionips* were dorsally dark brown or black, which contrasted with the ventrally pale yellow and white scale colour. This postpronotal scale colour pattern looked very similar to that of *Ae. rusticus* (Rossi, 1790). In males of *Ae. pionips*, the 2–4 anterodorsal scale rows of the postpronotum were golden yellow and similar to the scales on the scutal fossa. In comparison, all postpronotal scales of *Ae. communis* were yellowish-white, yellowish-brown, or, in some females, light brown on the dorsal third, but usually not dark and did not contrast with pale scales (Table 2). In some *Ae. communis* females with small body size and features overlapping with that of *Ae. pionips*, scales on the dorsal third of the postpronotum were darker, and the base of the costa possessed only several white scales. Wood et al. (1979) reported that subarctic specimens of

Table 2. Diagnostic characteristics for the differentiation between females and males of *Aedes communis* and *Aedes pionips*.

Character	*Aedes communis*	*Aedes pionips*
Female and male		
Hypostigmal scales	Absent (rarely 3–4 scales)	Absent
Scutal scales	Pale yellow, pale yellowish white, pale yellowish brown or light brown to sometimes lateral white; submedian stripes brown, narrow and broadly separated from each other (equal in width as a submedian stripe, posteriorly widened)	Golden pale yellow; submedian stripes black or dark brown, broad, and separated by a narrow pale acrostichal stripe (anterior single or double scale row, posteriorly widened)
Postpronotal scales	Pale yellow, light brown or pale yellowish white to white	Dorsally black or dark brown (of varying extent) to ventrally pale yellow, yellowish white or pale white
Postprocoxal scales	Absent	Present; few scales in males
Base of costa	Many white scales up to the humeral crossvein	Some white scales as a small patch (rarely several isolated white scales)
Mesonotal setae	All dark brown or medially brown to laterally light brown, yellowish brown or pale yellow (supraalar, scutellar); scutellar setae sometimes all dark brown or all pale yellow with few light brown setae	Medially black or dark brown to laterally and posteriorly yellow (all supraalar and scutellar setae); some pale setae darkened at base
Male		
Setae on ventral margin of basal dorsomesal lobe of gonocoxite	Strong and evenly curved	Thin, long and slightly curved; at base of lobe less curved; curved back apically (s-shape)
Claspette filament	Wing basally and medially broadened; apex shortly curved downwards	Wing basally broadened, medially flat; apex largely curved back

Ae. communis have scales along the dorsal edge of the postpronotum that are dark instead of yellowish-brown, a feature not observed for *Ae. communis* from the Erzgebirge or lower landscapes of Saxony.

The most reliable feature to distinguish adult *Ae. pionips* from *Ae. communis* are the postprocoxal scales, which are only present in *Ae. pionips* (Vockeroth 1954; Gutsevich et al. 1974; Becker et al. 2020). However, in some of the collected *Ae. pionips* specimens, only very few postprocoxal scales were present; considering that the scales can be lost, misidentification of specimens can happen. In lateral view, the pale basal bands on the terga of the abdomen of *Ae. pionips* appeared similar to those of *Ae. punctor*, although they had an almost straight median part which was narrowed and sometimes interrupted (few scattered black scales) in the middle of terga III–VII.

In previous descriptions of *Ae. pionips* from North America, it was noted that all or almost all scutal setae are dark brown to black, whereas in *Ae. communis*, all of these setae are either dark or yellow to bronze (Vockeroth 1954; Carpenter and LaCasse 1955; Wood et al. 1979). However, in *Ae. pionips* females collected in this study, the setae on the scutum were black or dark-brown, and yellow in the supraalar area and in the area posterior to the scutellum, a feature that was also observed by Gjullin et al. (1961) in Alaska. In the collected *Ae. communis* females, the colouration of mesonotal setae was similar as in *Ae. pionips*, but most of the scutal setae were dark. Also, *Ae. communis* females were found in which all mesonotal setae were dark brown.

It has been reported that the scales on the proepisternum, on the hind tibia, on the first tarsomere of the hindleg, and posterior to the metasternum (postmetasternal scales), as well as the tarsal claws of the foreleg, can be used to discriminate *Ae. pionips* from *Ae. communis* (Vockeroth 1952; Beckel 1954; Gunay et al. 2020). However, these features were not distinct or had a high variability in the specimens found in this study and, therefore, did not allow species discrimination.

The presence of postprocoxal scales in *Ae. pionips* males was the most reliable feature to discriminate this species from *Ae. communis*. However, some *Ae. pionips* males had only several or even no scales at all. In the previous literature, it has been reported that the palps of *Ae. pionips* males are either as long as the proboscis or slightly shorter, with their last segment being slender (Vockeroth 1952; Carpenter and LaCasse 1955; Wood et al. 1979). It was further reported that the palps slightly differ from those of *Ae. communis* males, which have palps longer than the proboscis, and the last segment being wider. Contrary to these reports, the palps of *Ae. communis* males collected in Saxony were either as long as the proboscis or even slightly longer. The palps of the *Ae. pionips* males were always longer than the proboscis: the length of palps is 1.03–1.10 times the length of proboscis (n = 8). Furthermore, the previously reported difference in the size of the last palp segment of male specimens was indeed observed. However, the difference was too small for unambiguous species discrimination. The wing of the claspette filament broadened at its base, both in *Ae. pionips* and *Ae. communis* (Gjullin et al. 1961) but appeared flat in *Ae. pionips* and broad in *Ae. communis*. Another distinct difference was seen in the curved tip of the claspette filament, which was longer and strongly recurved in *Ae. pionips*, when compared to *Ae. communis* (Table 2). For further morphological differences, see Table 2. Danilov (1984) stated that the length and shape of setae on the apicodorsal lobe of the gonocoxite are the most important features to discriminate *Ae. pionips* from *Ae. communis*, while Gjullin and Eddy (1972) reported that there are no differences between these features in the two species. In the specimens of the present study, no distinct difference in the setae on the apicodorsal lobe was observed between *Ae. communis* and *Ae. pionips*.

In the fourth-instar larval stage, *Ae. pionips* was differentiated from *Ae. communis* and *Ae. pullatus* by the characteristics of the cranial setae 5-C and 6-C, the thoracic setae 1-P, 1-M, and 3-M, and the comb scales. Characteristics found in this study to be useful for morphological species discrimination are given in Table 3. The most reliable feature for distinguishing *Ae. pionips* from *Ae. pullatus* larvae was the length of the mesothoracic setae 3-M and 1-M (Fig. 6). The larvae of *Ae. pionips* had a large body size and prominent black and spread fin setae, which made the larvae readily noticeable during collection from larval habitats and in the sampling jars.

The COI sequences of the two collected specimens (female and male) had highest nucleotide sequence identity with an *Ae. pionips* specimen from the United States (98.3% identity to BOLD accession number MOSN7454-21) and from Canada (98.2% identity to GenBank accession number JN302862.1), supporting their morphological identification as *Ae. pionips*. The two generated COI sequences, which were 100% identical, were deposited in GenBank under the accession numbers

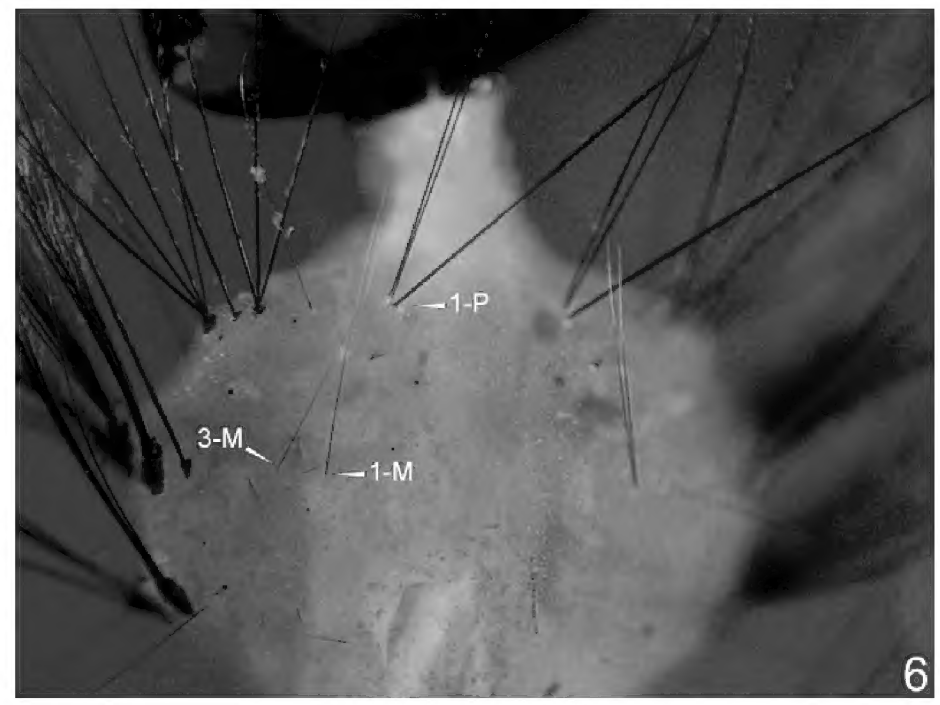


Figure 6. Dorsal view of the thorax of a fourth-instar larva of *Aedes pionips*; 1-P: prothoracic setae 1; 1-M: mesothoracic setae 1; 3-M: mesothoracic setae 3.

Table 3. Diagnostic characteristics for the differentiation between larvae of *Aedes communis*, *Aedes pionips* and *Aedes pullatus*.

Character	*Aedes communis*	*Aedes pionips*	*Aedes pullatus*
Cranial setae 5-C	Single	2–6 branches	3–7 branches
Cranial setae 6-C	Single	3–6 branches	3–8 branches
Prothoracic setae 1-P	2 branches	Single	2 branches
Mesothoracic setae	3-M more than twice as long as 1-M	3-M nearly as long as 1-M	3-M half to two thirds as long as 1-M
Comb scales	35–80 scales; apex with a fringe of stout spines of equal length which sometimes extend to the lateral margin	Usually 61–78 scales (rarely fewer than 60); oblong, small base, and long apically broadened apex with a fringe of sometimes asymmetrical, short spinules	40–60 scales; length of base equal to length of apex, which shows a large median spine

OM349596 and OM349597. The evolutionary relationship of the generated COI sequences was inferred by constructing a phylogenetic tree (Fig. 7). In this tree, the COI sequences of collected *Ae. pionips* specimens clustered with other *Ae. pionips* sequences, which further supported the morphological identification. The collected *Ae. pionips* specimens formed a distinct branch within the *Ae. pionips* cluster, which was supported by a 100% bootstrap value, reflecting the geographical separation of specimens from North America from those collected in Germany.

Larval habitats. The water level of the first larval habitat reached about 50 cm on 19 June. During the second survey on 18 July, the water levels of the first and the second

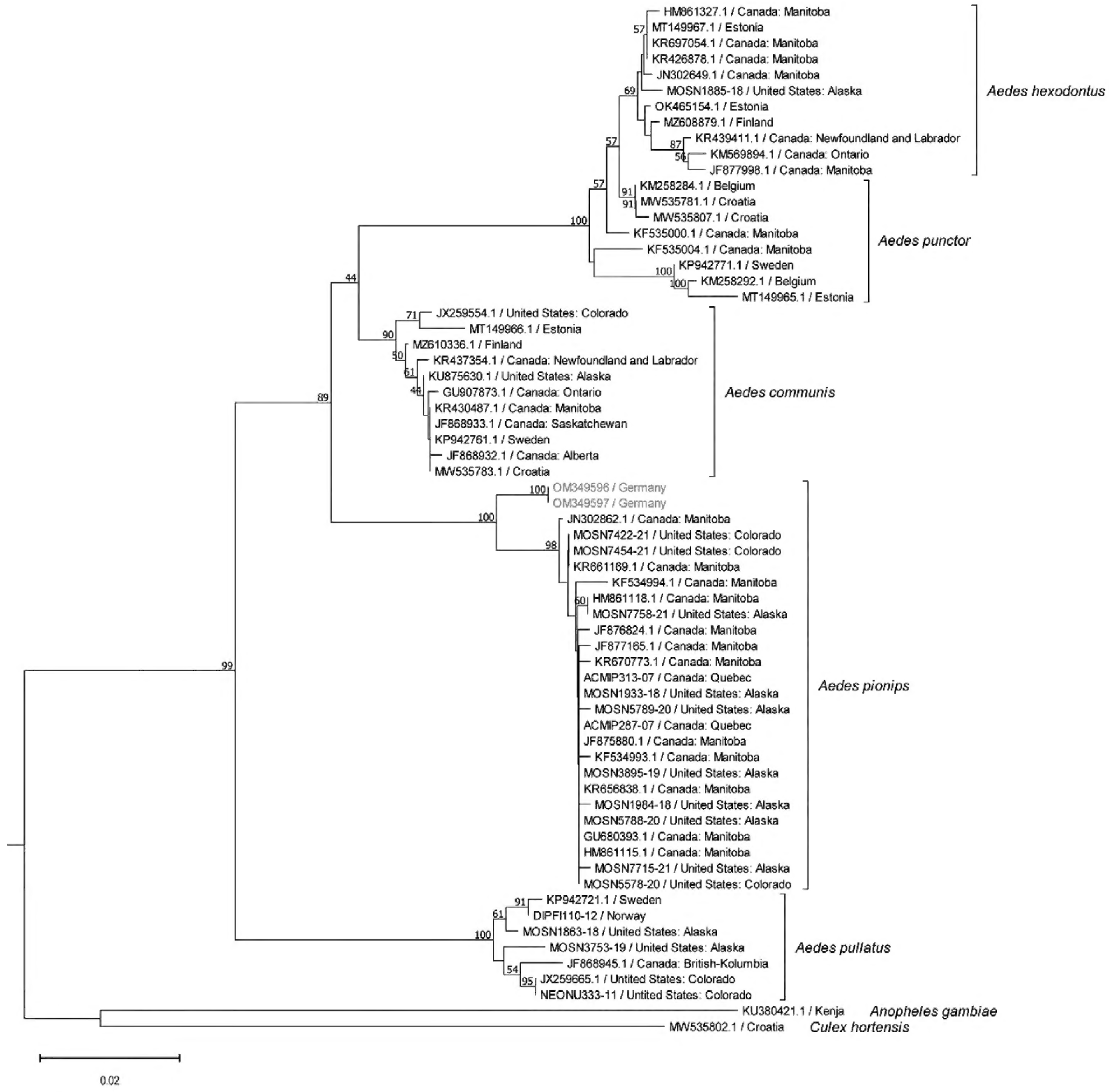


Figure 7. The identity of *Aedes pionips* specimens from Germany (in red) was supported by a phylogenetic tree using COI sequences. The tree was generated with the neighbour-joining method and 1,000 bootstrap replicates. Bootstrap values <44% were omitted. Branch lengths are proportional to the evolutionary distance and reflect the number of base substitutions per position. Branches show the accession numbers and country of origin for each specimen.

larval habitat reached about 80 cm. The water temperatures varied between 14 °C and 15 °C, which were measured at midday and in the evening. The two larval habitats were relatively large and deep when compared to neighbouring aquatic habitats, which were smaller and had a depth of about 10 cm, and temperatures that varied between 17 °C and 19 °C. Larvae of *Ae. pionips* were not found in the smaller aquatic habitats. During larval rearing, all larvae of *Ae. pionips* sampled in June died, whereas all specimens sampled in July emerged, except for one larva.

Discussion

The peatlands of the Erzgebirge are considered to be the western extension of the taiga peatlands of northwestern Russia and have a boreo-montane climate (Platen 1994; Edom and Keßler 2006). The aquatic habitats in and around the Georgenfelder Hochmoor in Germany as well as in and around the Seeheide-Zinnwald bog (Rašeliniště U jezera - Cínovecké rašeliniště) in the Czech Republic have been degraded or lost in the last decades (Freistaat Sachsen et al. 2006). Therefore, it is likely that *Aedes pionips* did not expand its distribution range to Germany recently, and that it is a rare and easily overlooked species even in its few remaining preferred habitats (Jenkins and Knight 1952). In all probability, it has been overlooked or misidentified in the past. The record of *Ae. pionips* presented in this study supplements other recent reports on very rare mosquito species that are native to Germany (Krüger and Tannich 2013; Kuhlisch et al. 2017, 2018). *Aedes pionips* can probably be found also in other locations in Germany if targeted surveys were carried out in suitable habitats. In Germany, 52 mosquito species have been reported so far (Werner et al. 2020), which is now increased to 53 species by including *Ae. pionips*.

In this study, males were observed during the first half hour after sunset, when they appeared for swarming high in a forest clearing, as also reported by Dyar (1919). At the end of June 2021, water levels were low due to a drought, reaching about 50 cm in deep depressions. After heavy rainfalls in early July 2021, these depressions were filled to about 80 cm and numerous small depressions, a few centimeters deep, were built. In June and July 2021, larvae or pupae of *Ae. pionips* were only found in 50–80 cm deep depressions. In those depressions, it is very likely that stable low water temperatures prevailed. These deep larval habitats were preferred by *Ae. pionips* over shallow pools at the study site and could have provided the necessary conditions for the known long larval development under cool temperatures.

Larval rearing was found to be difficult, as already reported by Scholefield et al. (1981). One possible explanation could have been that water temperatures were too high during rearing (Dahl 1974). Therefore, the water was cooled to 14 °C for the second collection, which enabled the successful rearing of larvae. The water temperatures of the pools measured in this study were in accordance with the observations that larvae developed at low water temperatures between 8 °C and 14 °C in Kazakhstan, and between 12 °C and 17 °C in South Karelia, Finland (Dubitsky 1970; Belova et al. 2008).

In the mountain ridge areas of the Erzgebirge, only a few raised bogs exist that can provide potential larval habitats for *Ae. pionips*. Other raised bogs that are similar to the Georgenfelder Hochmoor (eastern Erzgebirge) exist in the central Erzgebirge, like the Mothäuser Heide, the Schwarze Heide, and the Kriegswiese (Schindler et al. 2008). Raised bogs are particularly widespread in the western part of the Erzgebirge (catchment area of the Mulde). In this area, well preserved peatlands exist, including the Großer Kranichsee, the Kleiner Kranichsee, and the Henneberger Hang near Carlsfeld, as well as the Woderich peatland north of Schöneck (Schindler et al. 2008). Therefore, peatland habitats that are suitable for *Ae. pionips* are scarce and occur only locally, which limits the species' distribution in the Erzgebirge. In Saxony, the endangered habitat type, mire spruce woods (91D4), has a distribution hotspot in the western Erzgebirge and is threatened by complete destruction (Freistaat Sachsen et al. 2006). In the future, it is expected that the mountain ridge bogs of the Erzgebirge will suffer from reduced precipitation (Freistaat Sachsen et al. 2006). This would also threaten the larval habitats of *Ae. pionips*. The investigated peatland near Zinnwald-Georgenfeld largely extends into Czech territory. Therefore, it can be assumed that *Ae. pionips* also occurs in aquatic habitats in the neighbouring Czech Republic.

It is suggested that *Ae. pionips* is a very rare species in Germany, with a regional distribution only. The presented record was confirmed morphologically and supported genetically. The thorough morphological investigation in this study resulted in an unambiguous identification of *Ae. pionips* specimens from the Erzgebirge. The comparison with existing sequences from genetic databases results in a maximum agreement of only 98.3%. Thus, it should be considered that the two specimens genetically examined could be, for example, a subspecies of *Ae. pionips*, a genetic variant, or a cryptic species. The result of the barcode-based cluster analysis and the associated phylogenetic tree support the classification to *Ae. pionips*. Such differences between morphological and barcode-based identifications can have various causes, including a high geographical genetic variability. There is a large geographical distance between the *Ae. pionips* populations in North America and in Germany. Public COI sequences of *Ae. pionips* from Europe have not been available so far that made an analysis of genetic intraspecific variation more difficult. Future studies may possibly lead to an explanation of the observed identification problem.

Despite the fact that the morphological identification is difficult, it is possible to identify single adult and larval specimens using the morphological characteristics described in this article. It is also challenging to find the species. The occurrence of *Ae. pionips* in the Erzgebirge

is the most western known distribution area in Central Europe. The peatlands of the Erzgebirge at altitudes above 800 m provide suitable habitats, so that the persistence of *Ae. pionips* in these mountains depends on the preservation of peatland habitats. Due to the limited occurrence in the Erzgebirge, *Ae. pionips* is expected to have no significant role in the transmission of pathogens in Germany.

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